

CLAIMS

1. Use of inhibitors of h-Prune cyclic nucleotide phosphodiesterase activity for the preparation of a medicament for prevention and treatment of tumour metastases characterised by an overexpression of h-PRUNE, said inhibitors being selected from the group consisting of a peptide having the following amino acid sequence NIIHGSDSVESAEKE (SEQ ID No 9); a peptide comprising the following amino acid sequence NIIHGSDSVESAEKE GGGYGRKKRRQRRR (SEQ ID No 10); vinpocetine, IC261 and derivatives, structural analogues and isomers thereof.
2. Use according to claim 1, wherein tumours characterised by an overexpression of h-PRUNE are breast carcinoma, sarcoma, neuroblastoma, prostate tumour, pancreatic tumour, colon carcinoma tumour, rectal tumour, medulloblastoma, epithelioma, hepatocarcinoma, cell T or cell B lymphomas, myeloma and melanoma, and pulmonary tumour.
3. Peptide comprising the following amino acid sequence: NIIHGSDSVESAEKEGGGYGRKKRRQRRR (SEQ ID No 10) characterised in that it is permeable.
4. Peptide comprising the following amino acid sequence: NIIHGSDSVESAEKE GGGYGRKKRRQRRR (SEQ ID No 10) and characterised in that it is permeable and it is an inhibitor of h-Prune cyclic nucleotide phosphodiesterase activity, for use in medical field.
5. Peptide having the following amino acid sequence: NIIHGSDSVESAEKE (SEQ ID No 9).
6. Peptide having the following amino acid sequence: NIIHGSDSVESAEKE (SEQ ID No 9) characterised in that it is an inhibitor of h-Prune cyclic nucleotide phosphodiesterase activity, for use in medical field.
7. Screening method for h-PRUNE-inhibiting compounds, comprising the following phases:
- a) selection of at least a phosphoesterase (PDE) inhibiting compound or derivative, structural analogue or isomer thereof;
 - b) administration of said at least one compound at concentration between 0,05 μ M and 10 μ M in a cell line overexpressing h-PRUNE, wherein said cellular line is MDA-C100 435 prune #4;
 - c) quantitative analysis of the cyclic nucleotide phosphodiesterase activity of h-PRUNE and/or analysis of cellular motility versus concentration of said at least one compound and chemo-attractant and

selection of compound able to inhibit said phosphodiesterase activity between the values from 0.01 to 1 pmol/min⁻¹/ug⁻¹ and/or inhibit said motility up to the attainment of the values between 200 and 1200 cells.

5 8.Screening method according to claim 7, wherein the quantitative analysis of step c) is carried out by hydrolysis tests of the c-AMP and/or c-GMP substrate.

9.Screening method according to claim 7, wherein the substrate is used at concentration between 0,008 µM and 1 µM.

10 10.Method for in vitro detection of h-PRUNE in a biological sample for metastases diagnosis of tumours characterised by an h-PRUNE overexpression by immunological assay, FISH analysis, Real-time PCR, in situ hybridization.

11.Method for in vitro detection of h-PRUNE according to claim 10, comprising the following steps:

15 a) bring into contact said biological sample with at least one anti-h-PRUNE monoclonal antibody;
b) detection of the antigen-antibody complex;
c) quantitative analysis of the antigen-antibody complex.

20 12.Method according to claim 11, wherein said biological sample is a tissue section or biological fluid.

13.Method according to any one of claims from 10 to 12, wherein said anti-h-PRUNE antibody is the monoclonal antibody able to recognise and bind selectively the h-PRUNE recombinant protein, characterised in that it belongs to the IgM immunoglobulin class and is produced
25 by 4G3/4 clone (deposited at the CBA in Genoa on 10/12/2004)

14.Method according to any one of claims from 10 to 13, wherein said anti-h-PRUNE antibody is labelled with a radioisotope, fluorescent molecule or enzyme.

30 15.Method for in vitro detection of h-PRUNE according to claim 11, wherein said detection and quantitative analysis of the antigen-antibody complex are performed by immunohistochemistry, immunoprecipitation, immunofluorescence, ELISA, immunoblotting analyses.

16.Method according to claim 10, wherein PCR Real time primers specific for h-PRUNE comprise the sequences:

35 5'-AGAGATCTTGGACAGGCAAAC-3' (SEQ ID No 1);
3'-CCATGTTGACACAGTCCAGGAT-5' (SEQ ID No 2);
or their complementary sequences.

17.Method according to claim 10, wherein the labelled probe for Real-time PCR or in situ hybridization comprise the oligonucleotidic sequence: CTGCATGGAACCATC (SEQ ID No 3) or its complementary sequence or the sequence wherein T is replaced by U.

5 18.Method according to claim 17, wherein said labelled probe for Real-time PCR is linear or circular one.

19.Method according to any one of claims 17 and 18, wherein said probe is labelled with at least one radioisotope and/or fluorochrome.

10 20.Method according to any one of claims from 17 to 19, wherein said probe is labelled with at least a fluorochrome at 5' and/or 3'.

21.Method according to any one of claims from 17 to 20, wherein said fluorochrome is 6-carboxyfluorescein.

15 22.Diagnostic kit for the detection of h-PRUNE in a biological sample for metastases diagnosis of tumours characterised by an h-PRUNE overexpression comprising at least one anti-h-PRUNE monoclonal antibody, or a pair of primers specific for h-PRUNE or labelled oligonucleotidic probe specific for h-PRUNE.

20 23.Diagnostic kit according to claim 22, wherein the tumours characterised by an h-PRUNE overexpression are breast carcinoma, sarcoma, neuroblastoma, melanoma.

24.Diagnostic kit according to any one of claims 22 and 23, wherein said anti-h-PRUNE antibody is characterised in that it belongs to the IgM immunoglobulin class and is produced by 4G3/4 clone (deposited at the CBA in Genoa on 10/12/2004).

25 25.Diagnostic kit according to claim 24, wherein said anti-h-PRUNE monoclonal antibody is labelled with a radioisotope, fluorescent molecule or enzyme.

26.Diagnostic kit according to claim 22, wherein said pair of primers specific for h-PRUNE comprises the sequences:

30 5'-AGAGATCTTGGACAGGCAAACCT-3' (SEQ ID No 1);

3'-CCATGTTGACACAGTCCAGGAT-5' (SEQ ID No 2);

or their complementary sequences.

27.Diagnostic kit according to claim 22, wherein said labelled oligonucleotidic probe for Real-time PCR or in situ hybridization comprises the oligonucleotidic sequence:

CTGCATGGAACCATC (SEQ ID No 3)

or its complementary sequence or the sequence wherein T is replaced by U.

28. Diagnostic kit according to claim 27, wherein said labelled oligonucleotidic probe for Real-time PCR is linear or circular one.

5 29. Diagnostic kit according to any one of claims 27 and 28, wherein said oligonucleotidic probe is labelled with at least one radioisotope and/or fluorochrome.

30. Diagnostic kit according to any one of claims from 27 to 29, wherein said probe is labelled with at least one fluorochrome at 5' and/or
10 3'.

31. Diagnostic kit according to claim 30, wherein the fluorochrome is 6-carboxyfluorescein.

32. Monoclonal murine antibody able to recognise and bind selectively the h-PRUNE recombinant protein, characterised in that it
15 belongs to the IgM immunoglobulin class and is produced by 4G3/4 clone (deposited at the CBA in Genoa on 10/12/2004).